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A PECULIAR FAILURE OF HEMOLYTIC ACTION IN A STRAIN OF HEMOLYTIC STREPTOCOCCUS

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While titrating by the tube method the hemolytic capacity of a series of streptococcus cultures of the beta hemolytic type, a peculiar absence of hemolysis was observed with one culture.

This strain, W 12, isolated from a case of pleurisy has been described by Williams,[1] Valentine and Mishulow,[2] and Mishulow.[3] It is unusual in many respects. In blood-agar pour plates, it forms a rosette or complex colony unlike most beta hemolytic streptococci. It has the characteristics of the beta type in that microscopic examination shows that the red cells about the colony are completely dissolved, although the hemolyzed zone develops slowly, being very narrow after 24 hours' incubation and increasing in diameter to 3 mm. after 48 hours. The relatively feeble hemolytic capacity of this strain was also shown by the general absence of demonstrable hemolysin in the supernatant broth of centrifuged broth cultures. After 68 hours' growth in glucose broth, it reaches a H-ion concentration of P_H 4.7, which is more acid than the reaction produced by the majority of strains of human origin.

With the strain W 12, 0.5 c c of an 18-hour broth culture failed to give hemolysis when mixed with 1 c c of 5% washed red cell (horse), while 0.4 c c of the culture plus 0.1 c c of sterile broth resulted in almost complete hemolysis of the red cells (table 1). Twenty-five other strains did not show this phenomenon; the result with strain 60 is representative of the hemolytic activity of these other strains and is

TABLE 1

THE FAILURE OF HEMOLYTIC ACTION BY STRAIN W 12

Broth culture, 18 hours....	0.5 c c	0.4 c c	0.3 c c	0.2 c c	0.1 c c	0
Sterile broth..............	0	0.1 c c	0.2 c c	0.3 c c	0.4 c c	0.5 c c
Red cells, 5% suspension...	1 c c	1 c c	1 c c	1 c c	1 c c	1 c c
Strain 60..................	Complete lysis	Complete lysis	Complete lysis	Complete lysis	Complete lysis	No lysis
Strain W 12................	No lysis	Lysis	Lysis	Lysis	Slight lysis	No lysis

Horse, sheep and rabbit cells gave the same results.

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[1] Jour. Immunol., 1921, 6, p. 62.

[2] Ibid., 1921, 6, p. 301.

[3] Ibid., 1921, 6, p. 329.

given in table 1 for comparison. Another strain having the same characteristics as W 12, but differing from it agglutinatively, also showed hemolysis in all the tubes.

The failure of the whole culture of strain W 12 to lake red cells until the addition of sterile broth is of interest from the practical standpoint, for it may be a source of error in testing the hemolytic activity by the tube method usually employed. From the theoretical standpoint it seemed of interest to explain, if possible, the mechanism of this phenomenon.

In most of the further experiments, 0.5 c c and 0.2 c c of culture were used. The phenomenon was observed with marked constancy without regard to the kind of broth employed, that is, whether made of veal or of beef, the mode of sterilization, the period of storage and the variation of reaction within the limits suitable for growth.

It was found, however, that 0.5 c c of a broth culture of W 12, which had been incubated for only 6 hours, caused hemolysis, without the addition of fresh broth, but that the same culture incubated for 15 or 18 hours did not (table 2). The failure of hemolysis by the 18-hour culture was always prevented by the addition of 0.1 c c of sterile broth, and the addition of as little as 0.03 c c of broth resulted in the development of some hemolysis.

TABLE 2

THE HEMOLYTIC ACTION OF CULTURES OF STRAIN W 12 INCUBATED ONLY SIX HOURS; ABSENCE OF HEMOLYSIS AFTER LONGER INCUBATION

Broth culture, 6 or 18 hours	0.5 c c	0.5 c c	0.2 c c	0.2 c c
Sterile broth	0	0.1 c c	0	0.1 c c
Salt solution	1.0 c c	0.9 c c	1.3 c c	1.3 c c
Red cells, 5% suspension	1.0 c c	1.0 c c	1.0 c c	1.0 c c
Results, 6 hours culture	Complete lysis	Complete lysis	Lysis	Lysis
Results, 18 hours culture	No lysis	Complete lysis	No lysis	Lysis

When the clarified supernatant broth of an 18-hour culture of W 12 was used in place of sterile broth in an experiment parallel to that shown in table 2, no hemolysis took place. Heating this supernatant broth to 56 C. and 100 C. for 15 minutes, gave no different results. The supernatant broth from an 18-hour culture when added to a 6-hour culture did not prevent hemolysis. Adding peptone water to 18-hour broth cultures was as effectual in renewing hemolytic activity as the addition of sterile broth. Sterile horse serum, however, did not serve so well. Acid production was evidently not a factor, for neutralization of the small amount of acid produced did not result in hemolysis.

The outstanding points were therefore: the active hemolysis after 6 hours' incubation, the absence of hemolysis after longer incubation, the renewal of hemolytic action by the addition of broth or peptone water, that is, a fresh food supply. This raised the question: Was the failure of hemolysis due to the cessation of growth and was the resumption of hemolytic action, after the addition of fresh broth, due to renewed multiplication?

This point was investigated by making poured plates. After 2 hours' incubation of the tube to which no fresh broth had been added, plate counts showed at most a slight increase in the number of cocci. The tube, however, to which fresh broth had been added, showed after 2 hours' incubation an increase of 30 to 60% in the number of colonies developing in the plates.

SUMMARY

It would seem that the failure of hemolysis of the strain of hemolytic streptococcus studied was due to cessation of growth which was renewed by the addition of a small amount of fresh broth. The reason, however, why this culture should present this peculiarity remains obscure.